ANNUAL REPORT

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Contract No. NAS 9 8200

July 1, 1968 through June 30, 1969

by

Lee R. Brown, B.S., M.S., Ph.D.
Member, University of Texas Dental Science Institute
Houston, Texas

Merrill G. Wheatcroft, D.D.S., Professor
The University of Texas Dental Branch
Houston, Texas

Sandra Allen, B.S., Research Associate
The University of Texas Dental Science Institute
Houston, Texas

TABLE OF CONTENTS

Forward	i
Introduction	1
Procedures	3
Results and Discussion	16
Summary	27
Tables 1 through 5	29 - 33
Figures 1 through 12	34 - 55

FORWARD

This is the first annual report on the project NAS 9 8200 entitled "Study to Define and Verify the Personal Oral Hygiene Requirements for Extended Manned Space Flight."

This research has been a cooperative effort between the University of Texas Dental Science Institute and the University of Texas Dental Branch.

The investigators which to acknowledge the following persons who have contributed greatly to the project. Dr. John Hampton, Dr. Don Hudson, and Mr. John Allen for their help in the design and fabrication of the hypobaric pressure chamber. Dr. Francis B. Gordon, Consultant, Naval Medical Research Insititue, Bethesda, Maryland, for technical and scientific advice. Dr. Samuel Dreizen for editorial suggestions. Mrs. Mary Fuller for secretarial assistance and Miss Barbara Hellmers for help in preparation of the illustrations.

STUDY TO DEFINE AND VERIFY THE PERSONAL ORAL HYGIENE REQUIREMENTS FOR EXTENDED MANNED SPACE FLIGHT

INTRODUCTION

The oral mucosa is among the first of the body tissues to show changes in many systemic diseases and stressful situations. Accordingly, any deleterious effects caused by prolonged isolation in manned spacecraft environment could be reflected intraorally either at the clinical and/or microscopic level.

Some of the environmentally related oral changes which might be expected to occur initially are alterations in gingival health, changes in oral microbial flora, and an increase in dental plaque. To detect these changes, clinical examinations, including gingival health indices and plaque scorings, should be made immediately before and after actual or simulated manned space flights.

Microbiologic examinations of the oral tissues can detect early changes which may not be apparent clinically. The oral microbial flora, therefore, should be studied in detail before, during, and after exposure of humans or animals to unnatural environments. Such examinations will provide a census of the microbial population resident in specific oral tissues of subjects at ambient conditions and in atmospheres simulating those of the manned spacecraft. Microbial population changes caused by unnatural atmospheric conditions, and the significance of these changes in leading to abnormal oral manifestations, can thus be evaluated.

Initially the proposed clinical and microbiologic examinations will

be evaluated in primates to determine their applicability to the human. Primates are preferred to other laboratory animals for this purpose since their dentition, oral flora, and oral diseases more nearly simulate those of man. The results of the primate studies will determine the practicability of the examination methods and the need of any necessary modifications.

The specific objectives of the present investigation are:

(1) To establish sampling methods that are adequate and practical for obtaining a microbial census of the intraoral tissues of humans and primates.

(2) To fabricate hypobaric pressure units suitable for housing small primates (marmosets) in a 70 per cent oxygen - 30 per cent nitrogen atmosphere at 5 PSI.

(3) To establish a baseline census of all cultivable oral microorganisms in marmosets housed under ambient conditions, and to determine any changes in the microbial flora during progressively increasing exposure to 70 per cent oxygen - 30 per cent nitrogen at hypobaric pressure (5 PSI).

PROCEDURES

I. Preliminary evaluation of microbiologic sampling, transport, diluting, and plating procedures.

A. Sampling Procedures

Since the oral specimens for the microbiologic study were to be obtained from both humans and small primates (marmosets - Sanguinus oedipus - Fig. 1a), it was necessary to develop suitable uncomplicated sampling methods. It was also desirable that the same sampling technic be applicable to both humans and marmosets. This problem was rather unique since the humans were to be isolated in the Life Support Systems Evaluator (LSSE) and the Control Activity Facility (CAF), (Wright-Patterson Air Force Base, Dayton, Ohio), and the marmosets maintained in hypobaric chambers (The University of Texas Institute for Dental Science, Houston, Texas).

Preliminary comparisons of methods for collecting saliva were made using swabs, wire loops, and paper points. Calcium alginate swabs (Calgiswab, Code No. 11-60E)[a] were tested in humans and marmosets. The swabs were weighed on a Mettler analytical balance (Model #H20)[b] before and after sampling to determine the quantity and reproductibility of saliva collection by this means. Quantitative and qualitative microbiologic analyses were performed to determine the type and number of recoverable microorganisms.

---

a/Colab Laboratories, Inc., Chicago Heights, Illinois.
b/Mettler Instrument Corp., Highstown, New Jersey.

Stainless steel wire loops contoured from orthodontic wire (#007 - diameter of 0.007 inches)[a] were evaluated as a means of collecting a defined volume of saliva. The wire was cut into strips 40 mm in length, bent around a 2 mm rod and twisted to form a 2 mm diameter loop. The shaft of the wire loop was inserted into the end of a glass capillary tube (2 mm O.D., 100 mm long) to a depth of 5 mm and fixed in place with red stick compound (Fig. 1b). Approximately 30 mm of the opposite end of the glass capillary tube was plugged with cotton and etched to provide a breakable handle. The loop was then sterilized with ethylene oxide and filled with saliva from beneath the tongue of humans and marmosets. The amount of saliva collected by the wire loop was determined on a weight basis using a Mettler analytical balance (Model #H20)[b].

Endodontic paper points (Absorbent Paper Points #15)[c] cut into 2 mm or 3 mm lengths and mounted in glass capillary tubes (Fig. 1b) were also used to collect and study the salivary microflora.

In addition to the swab, wire loop and paper point technics, human saliva was obtained following stimulation by chewing on rubber bands or paraffin sticks. Comparisons were made of the type and number of microbes in the samples collected by each method.

Wire loops and paper points were also tested for their feasibility as collection methods for crevicular fluid from the

a/Obtainable from most dental supply companies.
b/Mettler Instrument Corp., Highstown, New Jersey.
c/Union Broach Co., Inc., Elmhurst, New York.

gingival sulcus of man and marmoset.

Since toothbrushes were to be used and stored for extended periods by the men isolated in the LSSE and CAF units, toothbrush bristles were sampled at various time intervals after use as a source of residual microorganisms. Both natural and nylon bristles were randomly selected from different toothbrush sites at 3, 6, 12, and 24 hours after brushing to determine the type and number retained viable oral microorganisms. The bristles were removed aseptically and immersed in 2 ml of diluting medium for microbial analyses.

B. Transport Procedure

Logistical problems steming from the need to procure and transport specimens from Dayton, Ohio, to Houston, Texas, prompted the screening of different types of transport or maintenance media which would least alter the specimens prior to analysis. Buffered saline with and without 0.1 per cent agar, 0.01 per cent peptone with and without agar, and 0.1 per cent peptone in 0.85 per cent saline were compared as holding media for specimens exposed to 25°C, 4°C, and ± 0.05° (ice bath) for 1, 3, 6, and 18 hours.

C. Dilution and Plating Procedures

The media evaluated for transportation and maintenance of specimens were also assessed as sample diluents. Serial 10-fold dilutions of the specimens were made by adding 1 ml, 0.2 ml, and

0.1 ml aliquots to 9 ml, 1.8 ml, and 0.9 ml of transport media respectively. Microbiologic platings were made repeatedly in triplicate from each diluted specimen to determine the most accurate and reproducible procedure.

Microbiologic plating technic using agar overlay or a glass rod spreader were compared for colony distribution and reproducibility. Various enrichment and selective media were evaluated for suitability and practicability. Five per cent horse blood agar, 10 per cent horse blood agar, and Trypticase Soy Agar[a/] were tested for the recovery of total aerobic and total anaerobic microorganisms. Selective media for bacteroides, fusobacteria, streptococci, staphylococci, and enteric bacilli were also studied for suitability.

Human specimens for the preliminary evaluations were obtained, for the most part, from cancer patients at the University of Texas M. D. Anderson Hospital and Tumor Institute. The reason for using these patients was the opportunity to study the effects of a restricted environment on the human oral microflora. The group included patients in the usual hospital environment, and patients confined to sterile isolators (laminar air flow rooms and life island units [plastic isolator]). All food, water, bedding, etc., entering these units were sterilized before use. Such patients provide a unique opportunity to monitor microbial changes in a

a/DIFCO Laboratories, Detroit, Michigan.

protected environment roughly similar to the LSSE or CAF environments.

The findings of the preliminary studies are detailed in the result section of this report. On the basis of these evaluations the following procedures were adopted:

1. The use of wire loops for saliva specimens and 3 mm paper points for gingival sulcus specimens in both man and marmosets.
2. The chewing of sterile rubber bands for the collection of stimulated human saliva.
3. The use of calcium alginate nasopharyngeal swabs for oral swabs of marmosets.
4. The use of 0.1 per cent peptone in saline for the transport and dilution of wire loop, paper point, and swab specimens. The use of ice water bath to control the temperature during transport.
5. The use of serial 10-fold dilutions from 0.2 ml sample in 1.8 ml dilution media.
6. The use of glass rod spreaders for dispersion and plating of appropriate dilutions in duplicate.

The following media were selected for the isolation and enumeration of the specific categories of human and marmoset oral microorganisms.

Total aerobes - Heart Infusion Agar[a] plus 5 per cent defibrinated horse blood incubated three days at 37°C. (All aerobic plates were incubated in this manner.)

Total anaerobes - Heart Infusion Agar[a] plus 5 per cent defibrinated horse blood incubated 45 days at 37°C in anaerobe jars containing 95 per cent nitrogen and 5 per cent carbon dioxide. (All anaerobic plates were incubated in this manner.)

Streptococci - Mitis Salivarius Agar[a] incubated aerobically.

Salt Tolerant Staphylococci - Staphylococcus Medium 110[a] incubated aerobically.

Lactobacillus - Rogosa SL Agar[a] incubated aerobically.

Bacteroides - Heart Infusion Agar[a] plus 100mcgm/ml Kanamycin[b] 15 per cent laked blood, 0.1 per cent sodium bicarbonate, 0.5mcgm/ml menadione, and 7.5mcgm/ml vancocin[c] incubated anaerobically.

Fusobacterium - Trypticase Soy Agar[d] 4mcgm/ml crystal violet, and 7.5mcgm/ml vancocin[c] incubated anaerobically.

Veillonella - Veillonella Agar[a] incubated aerobically.

Enterics - Both Desoxycholate Agar[a] and Levine Eosin Methylene Blue Agar[d] incubated aerobically.

PPLO - PPLO Agar[a] with additives as described by Shklair and coworkers[e] and incubated anaerobically.

---

a/DIFCO Laboratories, Detroit, Michigan.
b/Bristol Laboratories, Syracuse, New York.
c/Eli Lilly and Company, Indianapolis, Indiana.
d/Baltimore Biological Laboratories, Baltimore, Maryland.
e/Shklair, I. L.; Mazzarella, M. A.; Gitekunst, R. G.; and Kiggins, E. M.: Isolation and Incidence of Pleuropneumonia-like Organisms from the Human Oral Cavity. J. Bact. 83:785-788, 1962.

All of the above media were used in assaying the human stimulated saliva and the oral marmoset swab samples. The loop samples of human and marmoset saliva were plated only for total aerobes, anaerobes, and streptococci as the dilution was too high to detect any other specific group of organisms. The human and marmoset gingival sulcus samples taken with a 3 mm paper point were plated on the same media as used for the loop specimens plus media for the cultivation of bacteroides, fusiforms, veillonella, and enterics. Differential counts of the predominating types of cultivable organisms were made from blood plates containing 50 to 100 colonies.

The categories of microbial counts and procedures for isolation and identification of the predominating flora are shown in the photographs of data sheets I, II, and III (Fig. 2).

II. Fabrication of a hypobaric pressure chamber to study the effects of simulated spacecraft environments on the oral health of small primates (marmosets).

Two plastic isolators (18" diameter and 36" long) were fabricated[a/] to house two marmosets each. The cylinder portion of the isolators was made from 3/8" acrylic plastic and endpieces from 1" acrylic plastic. The endpieces were held in place by six 3/16" diameter joining brass rods. The rods were threaded to permit tightening of the endpieces by wing nuts. A rubber gasket was recessed in each endpiece to affect a seal.

a/Plastic Fabrication, Inc., Houston, Texas.

The assembled chamber is shown in Fig. 3b and c. The plastic germ-free isolator used as prechamber and postchamber housing for study at ambient conditions is shown in Fig. 3a.

Cage racks to fit in the chamber were built from 1/4" acrylic plastic.[a] They were constructed to allow the caging of two marmosets in separate compartments (Fig. 4a). The ends and center dividers of the cage racks were perforated with numerous 1/2" holes for circulation of gasses. The base was formed of 1/4" plastic strips set 3/8" apart for passage of animal wastes (Fig. 4b). The wastes were collected on a removable 1/8" thick plastic plate (11" wide and 35" long) bent to direct urine flow toward the center. Wood shavings, silica gel, and anhydrous calcium chloride granules were placed on the plate to demoisturize the excretory products. Boric acid granules were added to inhibit bacterial degradation and Purafil[b] granules were included to absorb organic compounds.

Food boxes (6" long x 2-3/4" wide x 2" deep), water troughs (9" long x 2-1/4" wide x 2-3/8" sloping to 2" deep), and environmental control boxes (9" long x 5" wide x 2-15/16" deep), were made from 1/8" plastic and attached to the cage rack by bolts and wing nuts (Fig. 4c). The food boxes were filled with Purina Monkey Chow[c] ground approximately 1/4" diameter pellets.

The environmental control boxes contained chemicals for air purification, carbon dioxide scrubbing, and chamber dehumidification

---

a/Fabricated at the University of Texas Dental Science Institute, Houston, Texas.
b/Marbon Chemical, Division of Borg-Warner Corp., Washington, West Virginia.
c/Ralston Purina Co., St. Louis, Missouri.

during operation (Fig. 4c and d). Purafil[a/] (activated alumina-Al_2O_3 impregnated with potassium permanganate) was used for air purification and odor control (Fig. 4d). This absorbs and chemically oxidizes odors from organic acids and ammonia derived from the waste products. Carbon dioxide was removed by Baralyme granules[b/]; Tel-Tale Silica Gel[c/] dessicant with indicator added was used as a dehumidifier.

A stainless steel external unit was fabricated[d/] to recirculate chamber atmosphere and to remove carbon dioxide, water vapor, and organic contaminants (Fig. 5a). This unit was designed to circulate the atmosphere at 2 CFM by a magnetically driven squirrel cage blower. The blower circulated the chamber gases sequentially through a canister containing Baralyme, a canister of Silica Gel and Purafil over an electrode for monitoring the partial pressure of oxygen and back into the chamber (Fig. 5a and b). Various by-pass or shut-off valves were included in the recirculation system to control the flow through specific canisters, and to by-pass certain parts of the unit. This permitted recharging and calibrating of the oxygen electrode in the oxygen gland assembly and replacement of the pruification canister (Fig. 5a).

The oxygen level was continuously monitored by a Beckman Model 778 Process Oxygen Analyzer[e/] containing a polargraphic oxygen sensor. This sensor was placed into a gland assembly of the recirculation system as indicated in Fig. 5b. The concentration of other gases in the chamber, notably NH_3, H_2S, and CO_2 were checked periodically with a Unico

---

a/Marbon Chemical, Division of Borg-Warner Corp., Washington, West Virginia.
b/National Cylinder Gas, Chicago, Illinois.
c/Davison Chemical, Baltimore, Maryland.
d/Fabricated by Worldwide Development Corp., Columbus, Ohio.
e/Beckman Instruments, Inc., Fullerton, California.

Model 400 Precision Gas Detector.[a/]

Pure oxygen or a mixture of 70 per cent oxygen-30 per cent nitrogen was supplied to the chamber from 1A size cylinders[b/] of compressed gas provided with two-stage regulators (Fig. 5b).

Oxygen was supplied automatically to the chamber, as needed, to maintain a 70 per cent-30 per cent oxygen-nitrogen ratio at a chamber pressure of 5 PSI. Chamber pressure controlled the amount of oxygen supplied since the chamber and the recirculation unit were built as a closed (leak-proof) system maintaining a constant pressure. Any change in pressure such as caused by the uptake of oxygen during respiration (the conversion of oxygen to carbon dioxide which was removed by Baralyme) could be utilized as an oxygen controller. The assembly was fitted with a device designed and built at the Dental Science Institute to provide oxygen to the animal chamber when such need was indicated by an increase of $\pm$ 0.1" Hg of negative pressure (Fig. 6a). The unit consisted of a "V"-shaped mercury manometer (3/16" bore) formed from a four-inch square block of acrylic plastic (Fig. 6a and b), a 6 to 8 volt auto light bulb activated at 5 volts from a 1-volt filament transformer[c/] of standard design, a 10X microscope eyepiece to concentrate light, a Raytheon CK122 Photocell[c/], a small transistorized amplifier[d/], and a E4F1000S SPDT relay[c/] (Fig. 6b).

When the connection tube on the end of the manometer was clamped shut an increase in negative pressure moved the column of mercury

a/Union Industrial Equipment Corp., Fall River, Massachusetts.
b/Olin Mathieson Chemical Corp., La Porte, Texas.
c/Sterling Electronics, Houston, Texas.
d/Fabricated at the University of Texas Dental Science Institute, Houston, Texas

allowing light to pass through a 1/8" diameter recess in the translucent manometer block normally (at 5 PSI) obliterated by the mercury. The light activated the photocell and the impulse was amplified to control the Dormeyer Commercial solenoid type 4X241[a] (Fig. 6c). The solenoid then opened a tubing clamp[b] (Fig. 6c) which functioned as an oxygen valve.

III. Operational procedures for the hypobaric pressure chamber.

All items necessary for animal housing and atmosphere control were placed in the chamber and recirculation unit prior to caging the animals in the apparatus. The oxygen electrode was chemically charged with an oxygen electrolyte gel and a teflon film according to the manufacturer's instructions. The oxygen electrode was calibrated to a partial pressure of 160 mm Hg of oxygen at ambient conditions before insertion into the gland assembly of the recirculation unit.

The complete hypobaric assembly was next tested for maintenance of constant pressure by evacuation of 28.5 Hg of the chamber air. Air was exhausted by an oiless, greaseless vacuum pump (Gast)[c] attached to the recirculation unit by a three-way glass stopcock. At various pressures during the evacuation procedure the calibration of the oxygen electrode was rechecked for the calculated partial pressure of oxygen.

Upon establishing a leak-proof system, the chamber was repressurized to ambient conditions with the addition of room air. One end of the chamber was then opened to pass in the test animals.

The operation of the hypobaric chamber was tested by housing rats

---

a/Sterling Electronics, Houston, Texas.
b/Fabricated at the University of Texas Dental Science Institute, Houston, Texas.
c/Gast Manufacturing Corp., Benton Harbor, Michigan.

in the chamber at an atmosphere of 70 per cent oxygen and 30 per cent nitrogen at 5 PSI for one week. Immediately after inserting rats, approximately two-thirds of the air was evacuated from the chamber and replaced with pure oxygen to hyperventilate the animals and establish a 70:30 oxygen to nitrogen ratio. The chamber was reevacuated to 10" Hg to provide an atmosphere of 70 per cent oxygen and 30 per cent nitrogen (177 mm Hg oxygen and 76 mm Hg nitrogen) at 5 PSI. One connecting end of the "V"-shaped manometer was clamped off to allow the oxygen controller to operate in the presence of a change in pressure due to the removal of carbon dioxide.

On the fifth day of operation, the recirculation unit was closed off from the chamber and repressurized for the replacement of cannisters of air purifiers and the recharging of the oxygen electrode. Subsequently, the recirculation unit was completely evacuated and a mixture of 70 per cent oxygen and 30 per cent nitrogen was manually added to bring the unit to 5 PSI by means of a three-way stopcock. The unit was then reopened to the chamber.

Since rats tolerated the one-week exposure without any apparent ill effects, three-day pilot studies were run using first one marmoset and then two marmosets (Fig. 7). The marmosets proved to be more sensitive to oxygen depletion than rats. Because of this, it was necessary to evacuate and add oxygen in three steps to obtain the final chamber environment.

Following the pilot studies with the marmosets in the hypobaric chamber, the animals were removed and replaced into the germ-free isolator for readjustment to ambient conditions.

After a one-week readjustment, the animals were sampled microbiologically at two-day intervals for eight days to obtain four baseline (prechamber) counts prior to a two-week chamber isolation. During the fourth sampling period, the animals were examined, weighed, and placed in the chamber which was adjusted to the designated atmospheric conditions (Fig. 7). After the first week, the animals were removed, examined, weighed, sampled, and placed back into a clean chamber for another week. At the end of the second week the change over process was again repeated. After three days of readjustment, each animal was then sampled microbiologically to obtain four postchamber samplings.

Microbial counts from the prechamber and postchamber samples were then compared to the chamber counts.

RESULTS AND DISCUSSION

I. Results of microbiologic sampling, transport, diluting, and plating procedures.

Variability in weight between swabs necessitated weighing each swab before and after use in the oral cavity. This was particularly important because of the small weight of saliva and the oral debris which adhered to the swabs. The mean weight of 20 randomly selected swabs was 0.292 ± 0.010 gm before use with a 0.026 ± 0.007 gm increase after use. Consequently, swabs were used only in the marmosets to enumerate those oral microorganisms which were too few in number to be detected in samples collected by wire loops. Human whole saliva was collected by chewing rubber bands rather than by the swab method. Rubber bands were preferred because of the deleterious effect of ethylene oxide sterilization on the paraffin sticks.

The use of wire loops calibrated to collect 0.0005 ± 0.0001 ml of saliva was found to be a simple and reproducible method for sampling saliva. The main disadvantage was the inability to recover categories of the oral flora that occurred in small numbers. The wire loop method was inadequate for collecting gingival specimens because of the relatively minute volume of crevicular fluid available for sampling.

Endodontic paper points (3 mm) proved quite practical for collecting specimens from the gingival sulcus as they could be easily inserted and

they readily absorbed the crevicular fluid. There was, however, considerable variation in the quantity of specimen obtained by this method (mean of 0.0004 ml $\pm$ 0.0002 ml). Specimen size was often too small to permit recovery of the numerically small groups of micro-organisms.

Aside from simplicity and ease of collection, the greatest advantage of both the loops and paper points was that they could be used in both man and marmosets with equal facility to provide comparable saliva and gingival sulcus specimens.

The number of residual microorganisms recovered from the bristles of toothbrushes varied greatly from individual to individual and to a lesser extent between different samples from the same brush. Viable counts from the more heavily contaminated brushes (counts $> 1 \times 10^4$ bacteria/ml of bristle suspension) began to decrease within six hours and approached zero within 24 hours after use (brush replaced in plastic case at room temperature). Viable counts from lesser contaminated brushes (counts of 1×10^2 to 1×10^3/ml of suspension) started decreasing within three hours and approached zero as early as six hours after use. These time related reductions in residual micro-organisms may have been due to drying of the brushes. These results need to be substantiated by repeated sampling of several different individuals.

Evaluation of suitable transport, storage, and plating procedures

indicated that a medium consisting of 0.1 per cent peptone and 0.85 per cent NaCl produced the least change in viable counts of cultivable oral microorganisms from saliva.

No appreciable increases or decreases in viable counts from specimens in 0.1 per cent peptone and 0.85 per cent NaCl were found after three-hour storage at room temperature. Slight increases were noted after 18-hour storage at room temperature and slight decreases after 18 hours of refrigeration. In contrast buffered saline with and without agar produced diminutions in viable counts starting immediately and becoming more apparent with increasing storage time irrespective of temperature.

Microbial counts (particularly anaerobes) often increased in undiluted whole saliva held one to three hours at room temperature. The increases were inhibited by immersing the undiluted saliva samples in an ice bath immediately after collection. In contrast, undiluted whole saliva maintained at refrigerator temperatures up to 18 hours showed a decrease in microbial count.

Assessments of the accuracy and reproducibility of the diluting and plating procedures indicated that 10-fold serial dilutions of the specimens could be made satisfactorily with 0.2 ml of specimen and 1.8 ml of diluting media when specimen volumes were limited. Reproducible counts were readily obtained by surface inoculation of duplicate agar plates with 0.1 ml inoculum dispersed with a sterile glass rod spreader.

Tests of suitable plating media for direct counts of several categories of oral microorganisms showed some selective media to be too inhibitory. Some were relatively nonselective and required continuous monitoring. The most troublesome were the selective media for streptococci, fusobacteria, and bacteroides.

Azide blood agar, incubated anaerobically, frequently produced higher counts of streptococci than Mitis-Salivarius Agar. It also permitted the distinction of different hemolytic groups of streptococci. The inhibitory activity of sodium azide appeared to vary, however, with storage causing variability in selectiveness. In contrast, Mitis-Salivarius Agar was much easier to prepare, always reacted consistently and appeared to be a more dependable selective medium. Since streptococci made up a large percentage of the oral flora, differential counts on blood agar were used to correct for any over-inhibitory effects of the selective medium on this group. Differential counts also were used to obtain estimates of some types of predominating organisms for which no selective media were available; i.e., diphtheroids.

Trypticase Soy Agar plus inhibitors gave a better recovery of fusobacteria than the more commonly used FM Agar [a]. Similarly, the medium used for species of bacteroides appeared to give a better yield than similar media containing other combinations of the same and different antibiotics (neomycin and paromomycin - Humatin).[b] Fusobacteria and bacteroides often occurred in numbers high enough to be included in

a/DIFCO Laboratories, Detroit, Michigan.
b/Parke Davis and Company, Detroit, Michigan.

differential counts on anaerobic blood agar.

After comparing Desoxycholate Agar, Levine's Eosin Methylene Blue Agar and MacConkey Agar[a/] for the isolation of enteric organisms from the oral cavity, it was decided to use both Desoxycholate and Eosin Methylene Blue Agar since each medium appeared to be inhibitory to different groups of enterics.

Although there were obvious disadvantages in using selective media for direct counts of specific groups of microorganisms, it does provide a means of monitoring changes in those which comprise small portions of a mixed population.

The results from preliminary baseline studies on the oral flora of marmosets are presented in Tables 1 and 2. Microbial counts from loop collected saliva at six sample periods (weekly intervals in most cases) are shown in Table 1. The total aerobes, anaerobes, and streptococci counts were the most numerous and most consistent in both marmosets. The next most prevalent organism, the fusobacteria, displayed extensive variability from one sample period to the next in each animal (Table 1). Counts of staphylococci and bacteroides, were periodically low and varied greatly between animals, as well as between different samples from the same animal (Table 1).

It was interesting that several categories of microorganisms commonly observed in human saliva were seldom or never found in the saliva of marmosets; i.e., veillonella, lactobacilli, candida, and myco-

---

a/DIFCO Laboratories, Detroit, Michigan.

plasma. Although several types of streptococci were isolated Streptococcus salivarius, not included as a category in the table, was never cultured from marmosets.

Microbial counts from the gingival sulcus area are shown in Table 2. Counts from this site were generally a little higher than those from saliva. The counts from Marmoset No. 1374 were usually a little higher and more varied than those from Marmoset No. 1658 (Table 2). This was particularly interesting since the latter animal had less calculus and more healthy appearing gingivae.

Some of these data converted to logarithms (base 10) for a more realistic comparison are shown in Fig. 8. Note the similarities in counts in the predominating categories of microorganisms in saliva and gingival sulcus specimens from each animal.

Counts of the most numerous organisms from loop collected marmoset saliva were compared to the most numerous microorganisms in loop collected saliva from a bed patient at M. D. Anderson Hospital receiving cancer chemotherapy without antibiotics (Fig. 9a). There was a relatively close numerical similarity in the comparable categories of microorganisms from each source.

Comparisons of the most numerous microorganisms cultured from human and marmoset gingival sulcus specimens are shown in Fig. 9b. Again the data reveals a similarity in the most numerous cultivable microorganisms.

Although the quantitative comparisons of the human and marmoset oral microflora were in close agreement, qualitative data derived from the isolation and identification of the predominating species, were in many instances completely different. This difference not only posed a problem in establishing differential counts (relative numbers) of the marmoset flora, but also species specific comparisons between marmoset and human impractical.

In an attempt to resolve this problem, unrecognized types of predominating species from marmosets were cataloged morphologically and biochemically. Between 50 to 60 such species have been identified in this manner to serve as a guide for future studies of population change in this primate.

II. Results of two-week exposure of marmosets to the hypobaric pressure chamber.

Two marmosets reacted to the pressure changes during the initial adjustment to 5 PSI by head shaking and scratching or pulling at their ears. One animal regurgitated each time the chamber was evacuated.

Both animals developed ophthalmic complications during their first three days in the chamber. Initially they rubbed or scratched their eyes, and blinked excessively. Later their eyes appeared glazed and dry and the eyelids became edematous. By the third day, the eyes of both animals were nearly swollen shut. The swelling subsided rapidly during the fourth day and by the fifth day the eyes appeared normal.

The most plausible explanation for the transitory eye involvement is the excessive dryness of the atmosphere induced by the fresh dessicating substance which later became moisturized.

By the end of the first week in the chamber environment, one animal (No. 1374) had lost 44 gm (424 gm to 380 gm) while the other (No. 1658) had lost 57 gm (410 gm to 353 gm). This rather extensive weight loss may be attributed to an unfamiliar environment, incomplete adjustment to the change in diet, and/or discomfort from eye involvement.

During the second week of exposure in the chamber the only abnormality was excessive hair loss. The eyes appeared quite normal and the animals ate more than during the previous week. The lack of eye involvement may have resulted from the accidental spillage of water from the watering troughs to the bottom of the chamber during the placing of the chamber on the stand. This could be significant if the dryness of the atmosphere was responsible for the eye envolvement.

Both animals regained more than half their weight loss during the second week: Marmoset No. 1374 gained 25 gm (380 gm to 405 gm) and No. 1658 gained 28 gm (353 gm to 381 gm). Each developed a relatively severe diarrhea following removal from the chamber. At the seventh postchamber day, they had regained an additional 8 to 10 grams despite the diarrhea.

Microbial counts from different types of oral samples obtained immediately before, during and immediately following the two-week

isolation period in the hypobaric pressure chamber are shown in Tables 3, 4, and 5.

Counts ($X10^2$) of the categories of the predominant microorganisms cultured from loop collected saliva are presented in Table 3. The prechamber counts are quite similar to the preliminary baseline counts shown in Table 1 in spite of the change from a moist, soft diet to a dry, relatively hard diet.

In comparing counts obtained before, during, and after exposure to the hypobaric chamber environment those made during and after exposure to the chamber appeared to be somewhat lower than the pre-chamber values. This was most apparent in the streptococcal counts (Table 3).

The above comparisons are presented graphically in Fig. 10. Although there appears to be a slight decreasing trend, the curves fall within the variation of the comparable preliminary baseline counts shown in Fig. 8.

Counts ($X10^3$) of the categories of predominant microorganisms cultured from paper point suspensions of gingival sulcus fluid are presented in Table 4. Again, there were some initial decrease in counts (fusobacteria and bacteroides) followed by an increase during the second week of chamber isolation. Streptococcal counts decreased steadily in one animal (No. 1374) during chamber isolation and returned to approximately the prechamber values after removal from the chamber. These changes can be seen more readily in the graphic counts ($\log_{10}$) (Fig. 11).

Microbial counts ($X10^1$/mg of debris) from oral swabs are listed in Table 5. Graphs of the counts ($\log_{10}$) are shown in Fig. 12. These data were derived from direct counts of microorganisms cultured on selective media. Counts of fusobacteria and bacteroides showed rather pronounced increases in both animals by the second week of chamber isloation. The increases persisted during the postchamber sampling period (one week).

The enteric counts of one marmoset (No. 1658) increased from zero to a relatively high level by the second week of chamber isolation (Fig. 12). This was of epidemiologic interest since the other marmoset (No. 1374) demonstrated a relatively high enteric count when placed into the chamber. The specific organism identified in each instance was Pseudomonas aeruginosa. The data was also indicative of a possible transmission of mannitol negative, salt tolerant staphylococcus from one marmoset to the other during isolation in the hypobaric pressure chamber (Table 5 and Fig. 12).

The significance of the microbial changes noted during the two-week chamber isolation is as yet equivocal in view of the variability in the categorical counts obtained from the preliminary baseline and prechamber samplings. These changes must be substantiated with additional studies involving chamber isolation of two weeks and longer. This need is also supported by the comparisons of the different types of organisms in the predominant aerobic and anaerobic oral flora of the marmosets. There

was a large variance in predominant types between animals as well as between different samples from the same animal. The most frequently cultured aerobes were species of Neisseria, Streptococcus, Corynebacterium, Staphylococcus, and unidentified gram positive and gram negative rods. The most frequently cultured anaerobes were species of Fusobacterium, Bacteroides, Corynebacterium, Streptococcus, and unidentified gram negative and gram positive rods.

SUMMARY

Adequate and practical sampling methods have been developed to obtain a microbial census of intraoral tissues of humans and marmosets. These methods utilize wire loops to collect saliva and paper points to collect gingival sulcus fluid from humans and marmosets. The methods permit comparable assays of the predominating microorganisms in specific areas of the oral cavity of man and marmoset.

Oral microbes occurring in relatively small numbers were assayed from swabs of the oral tissues of marmosets and from the saliva of humans collected by chewing sterile rubber bands.

The most suitable transport, diluting, and plating procedures for microbiologic analysis of the specimens were determined.

Preliminary baseline counts of cultivable oral microorganisms from marmosets housed under ambient conditions were established. The marmoset counts were found to be quite similar to human counts.

A relatively inexpensive hypobaric pressure chamber was fabricated which is suitable for studying the effects of simulated manned space-craft environments on the oral health of marmosets.

Two marmosets were maintained in the chamber for two weeks in an atmosphere of 70 per cent oxygen and 30 per cent nitrogen at 5 PSI. The adverse effects were excessive weight and hair loss, periorbital swelling, and severe diarrhea after removal from the chamber.

Microbial counts from oral samples collected immediately before,

during and immediately after chamber isolation showed both quantitative and qualitative changes. The significance of these changes awaits confirmation and substantiation by repeated and expanded studies.

Table 1

Microbial Counts of Loop Collected Saliva From Marmosets (Count X10^3/ml of Loop Suspension)

	Sample No.	1		2		3		4		5		6	
	Animal No.	1374	1658	1374	1658	1374	1658	1374	1658	1374	1658	1374	1658
Microbial Category	Total Aerobes	30.0	46.0	236.0	208.0	8.0	10.0	8.5	14.0	21.3	39.0	24.2	4.7
	Total Anaerobes	50.0	54.0	354.0	188.0	8.0	12.4	18.0	48.5	80.0	108.0	61.0	19.3
	Total Strep.	36.0	44.0	100.0	114.0	0.8	11.8	10.1	29.0	6.0	100.0	0.5	0.8
	Fusobacteria	0.0	0.0	8.8	0.2	0.0	0.0	1.0	2.0	1.4	0.0	0.7	0.2
	Bacteroides	0.0	0.0	57.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
	Veillonella	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0
	Salt. Tol. Staph.	0.0	0.6	0.0	0.2	0.2	5.6	0.0	0.0	0.0	0.0	0.0	0.2
	Enterics	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.6	0.0	0.0
	Lactobacilli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Candida	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	PPLO	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2

Microbial Counts From the Gingival Sulcus Area of Marmosets (Counts $X10^3$/ml of Paper Point Suspension)

	Sample No.	1		2		3		4		5		6	
	Animal No.	1374	1658	1374	1658	1374	1658	1374	1658	1374	1658	1374	1658
Microbial Category	Total Aerobes	56.00	92.40	800.00	22.40	3.00	21.40	28.40	49.00	63.80	40.00	420.00	20.30
	Total Anaerobes	67.80	100.00	800.00	34.40	65.40	32.00	800.00	109.00	160.00	74.00	800.00	79.00
	Total Strep.	8.60	100.00	------	11.20	8.00	8.00	80.00	26.80	3.00	20.20	3.20	1.30
	Fusobacteria	8.00	8.00	0.70	2.74	8.00	0.24	82.00	1.44	2.60	30.00	1.76	0.00
	Bacteroides	------	0.00	8.00	0.00	0.86	0.00	0.00	0.00	2.00	0.00	8.00	0.00
	Veillonella	0.00	0.00	0.50	3.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Salt. Tol. Staph.	0.00	0.02	0.00	0.00	0.00	0.06	0.00	0.12	0.00	0.00	0.00	0.04
	Enterics	0.00	8.00	0.00	1.48	0.00	5.42	0.00	0.18	0.02	2.00	0.00	0.00
	Lactobacilli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Candida	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	PPLO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3

Counts $X10^2$/ml of Loop Suspensions of Saliva

Microbial Category	Animal Number	Prechamber Samples				Chamber Samples		Postchamber Samples			
		1st	2nd	3rd	4th	1 wk	2 wks	1st	2nd	3rd	4th
Total	1658	7.20	11.76	20.33	23.30	3.60	2.70	3.60	8.70	3.00	10.20
Aerobes	1374	108.50	341.00	34.32	58.45	76.00	8.20	22.00	10.90	17.30	26.20
Total	1658	42.50	166.00	48.00	86.00	4.40	14.40	18.80	27.00	17.30	31.00
Anaerobes	1374	254.00	336.00	98.50	158.00	144.00	16.40	26.00	27.00	26.20	135.00
Total	1658	4.80	8.00	3.20	6.30	3.60	0.00	1.60	2.00	0.00	2.80
Streptococci	1374	17.40	62.00	2.00	24.00	24.00	1.20	3.40	0.60	13.00	12.80

Table 4

Counts $X10^1$/mg of Debris From Oral Swabs

Microbial Category	Animal Number	Prechamber Samples				Chamber Samples		Postchamber Samples			
		1st	2nd	3rd	4th	1 wk	2 wks	1st	2nd	3rd	4th
Salt Tol. Staph.	1658	0.00	46.36	1.00	53.78	0.72	10.00	1.20	38.00	50.00	17.33
	1374	0.00	0.86	0.18	0.00	0.00	2.50	0.50	0.50	9.09	16.00
Bacteroides	1658	165.46	0.00	0.00	16.22	43.64	466.67	120.00	0.00	540.00	15.33
	1374	445.46	28.50	0.00	220.00	35.56	2750.00	0.00	65.00	618.18	302.86
Fusobacteria	1658	563.64	0.00	0.00	77.78	0.00	1966.67	700.00	20.00	6400.00	13.33
	1374	145.46	11.29	16.36	21.77	55.56	916.67	1000.00	1600.00	272.73	285.71
Enterics	1658	0.00	0.00	0.00	1.34	0.00	11.00	1.20	4.00	260.00	2.33
	1374	0.73	5.86	3.82	0.00	0.00	0.33	1500.00	0.00	0.00	0.00
Veillonella	1658	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1374	0.00	0.00	0.00	3.18	0.00	0.00	0.00	0.00	0.18	0.00
Yeast	1658	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00
	1374	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5

Counts $X10^3$/ml of Paper Point Suspension From Gingival Sulcus

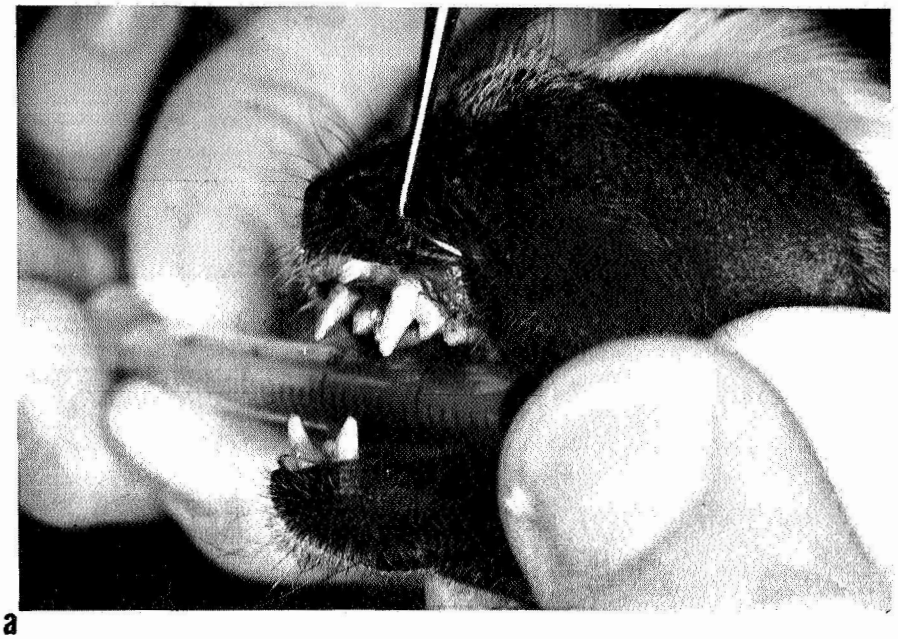
Microbial Category	Animal Number	Prechamber Samples				Chamber Samples		Postchamber Samples			
		1st	2nd	3rd	4th	1 wk	2 wks	1st	2nd	3rd	4th
Total Aerobes	1658	238.00	52.50	19.80	85.40	30.50	488.66	34.60	78.65	60.00	70.00
	1374	135.00	36.00	94.00	2040.00	59.34	35.96	22.00	80.00	19.40	80.00
Total Anaerobes	1658	425.00	326.00	52.66	790.00	100.00	305.00	270.00	320.00	106.00	214.00
	1374	725.00	480.00	218.00	1850.00	660.00	255.00	560.00	388.00	152.00	260.00
Total Streptococci	1658	30.60	2.30	0.18	0.98	3.02	6.38	4.80	0.50	1.64	0.32
	1374	16.00	3.28	0.90	5.00	1.24	0.08	0.50	13.40	1.28	4.32
Bacteroides	1658	0.20	0.00	0.00	0.00	0.00	0.00	0.00	2.04	0.00	0.00
	1374	8.00	0.00	0.00	46.60	0.00	43.00	6.00	2.00	12.00	0.10
Fusobacteria	1658	34.00	0.00	0.00	14.10	0.02	20.40	16.00	0.04	1.96	0.10
	1374	20.00	0.58	0.30	29.20	0.40	13.00	12.00	2.60	3.00	3.00
Enterics	1658	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
	1374	0.00	0.24	0.04	0.28	0.00	0.00	0.00	0.00	0.00	0.00

LEGEND FOR FIGURE 1

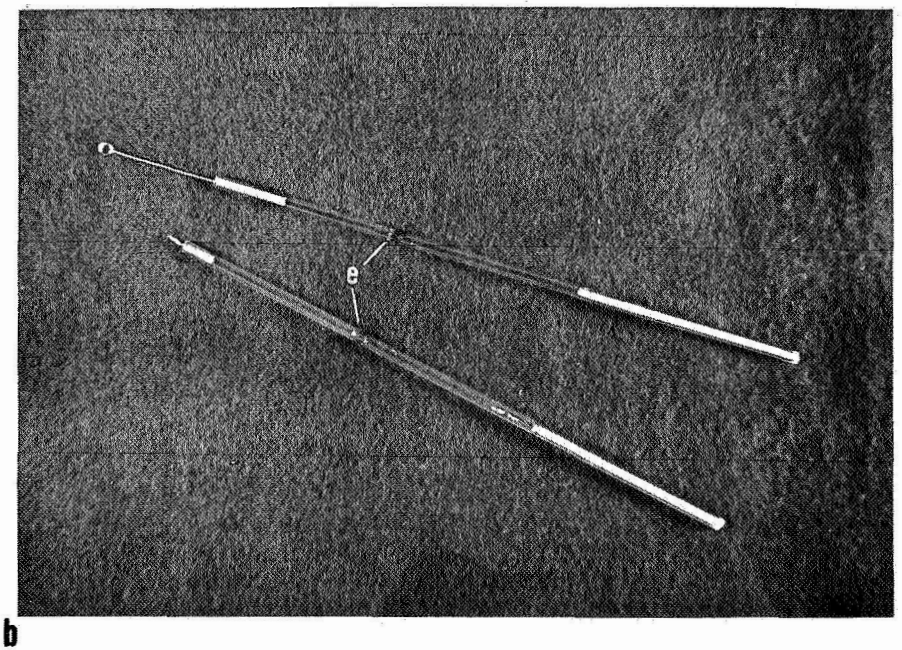
Method of collecting oral specimens from marmosets (Saguinus oedipus).

a. Oral cavity held open by a tuberculin plastic syringe. Lip retracted with a dental scaler to permit sampling from gingival sulcus. Deposits of calculus can be seen in the retracted area.

b. A wire loop (2mm) and a paper point (3 mm) attached to glass tubing handles for collecting saliva and gingival sulcus specimens respectively. The handles were etched (e) below the cotton plugs to facilitate breakage for aseptic transfer into test tubes.



a

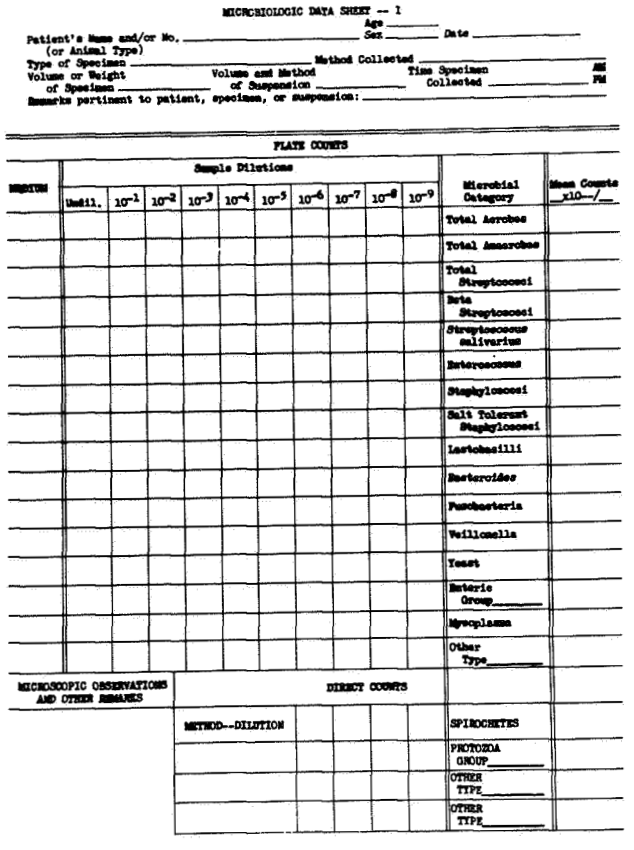


b

Figure 1

LEGEND FOR FIGURE 2

Data sheets used to record direct counts of microbial categories and morphologic and physiologic characteristics of the predominant microorganisms cultured on blood agar plates.

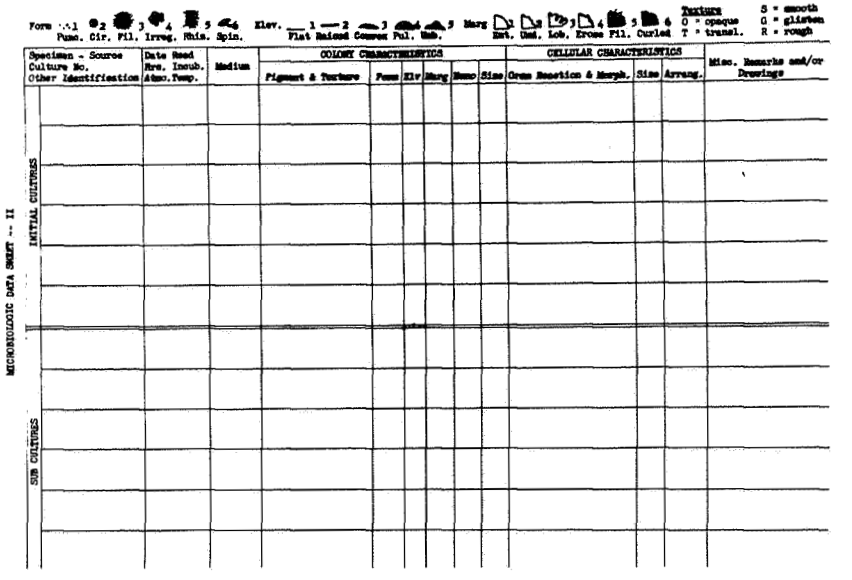


MICROBIOLOGIC DATA SHEET -- I

Patient's Name and/or No. _____ Age _____ Sex _____ Date _____
(or Animal Type)
Type of Specimen _____ Method Collected _____
Volume or Weight of Specimen _____ Volume and Method of Suspension _____ Time Specimen Collected _____ AM PM
Remarks pertinent to patient, specimen, or suspension: _____

REMARKS	PLATE COUNTS: Sample Dilutions Undil.	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	Microbial Category	Mean Counts _x10—/__
											Total Aerobes	
											Total Anaerobes	
											Total Streptococci	
											Beta Streptococci	
											Streptococcus salivarius	
											Enterococcus	
											Staphylococci	
											Salt Tolerant Staphylococci	
											Lactobacilli	
											Bacteroides	
											Fusobacteria	
											Veillonella	
											Yeast	
											Enteric Group_____	
											Mycoplasma	
											Other Type_____	

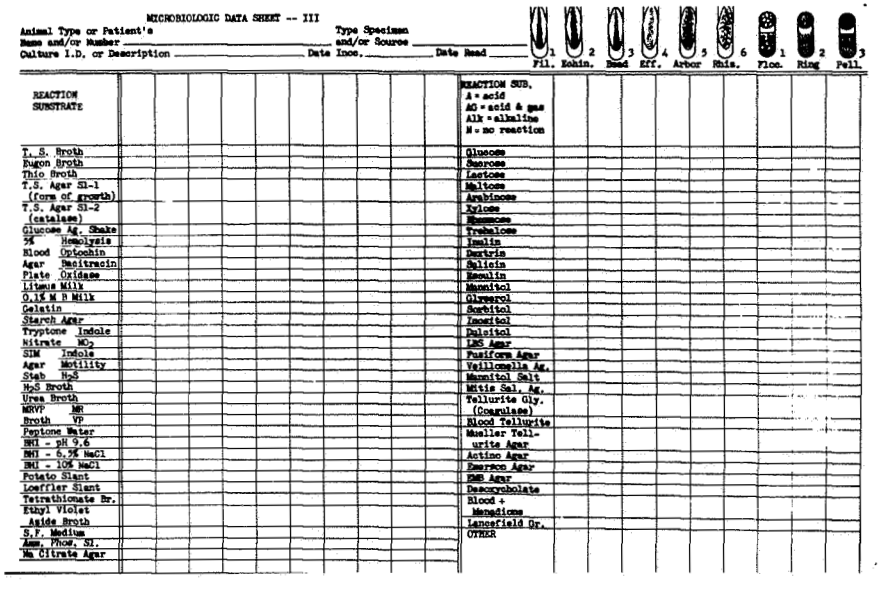
MICROSCOPIC OBSERVATIONS AND OTHER REMARKS	DIRECT COUNTS: METHOD--DILUTION						Microbial Category	Mean Counts
							SPIROCHETES	
							PROTOZOA GROUP_____	
							OTHER TYPE_____	
							OTHER TYPE_____	



MICROBIOLOGIC DATA SHEET -- II

Form: 1 Punc. 2 Cir. 3 Fil. 4 Irreg. 5 Rhiz. 6 Spin.
Elev.: 1 Flat 2 Raised 3 Convex 4 Pul. 5 Umb.
Marg: 1 Ent. 2 Und. 3 Lob. 4 Erose 5 Fil. 6 Curled
Texture: O = opaque T = transl. S = smooth G = glisten R = rough

	Specimen - Source Culture No. Other Identification	Date Read Hrs. Incub. Atmo. Temp.	Medium	COLONY CHARACTERISTICS: Pigment & Texture	Form	Elv	Marg	Homo	Size	CELLULAR CHARACTERISTICS: Gram Reaction & Morph.	Size	Arrang.	Misc. Remarks and/or Drawings
INITIAL CULTURES													
SUB CULTURES													



MICROBIOLOGIC DATA SHEET -- III

Animal Type or Patient's Name and/or Number _____ Type Specimen and/or Source _____
Culture I.D. or Description _____ Date Inoc. _____ Date Read _____

1 Fil. 2 Echin. 3 Bead 4 Eff. 5 Arbor 6 Rhiz. 1 Floc. 2 Ring 3 Pell.

REACTION SUBSTRATE	REACTION SUB. A = acid AG = acid & gas Alk = alkaline N = no reaction	
T.S. Broth		Glucose
Fugon Broth		Sucrose
Thio Broth		Lactose
T.S. Agar Sl-1 (form of growth)		Maltose
T.S. Agar Sl-2 (catalase)		Arabinose
Glucose Ag. Shake		Xylose
5% Blood Hemolysis		Rhamnose
Agar Optochin		Trehalose
Bacitracin		Inulin
Plate Oxidase		Dextrin
Litmus Milk		Salicin
0.1% M R Milk		Esculin
Gelatin		Mannitol
Starch Agar		Glycerol
Tryptone Indole		Sorbitol
Nitrate NO_2		Inositol
SIM Indole		Dulcitol
Agar Motility		LMS Agar
Stab H_2S		Fusiform Agar
H_2S Broth		Veillonella Ag.
Urea Broth		Mannitol Salt
MRVP MR		Mitis Sal. Ag.
Broth VP		Tellurite Gly. (Coagulase)
Peptone Water		Blood Tellurite
BHI - pH 9.6		Mueller Tellurite Agar
BHI - 6.5% NaCl		Actino Agar
BHI - 10% NaCl		Emerson Agar
Potato Slant		EMB Agar
Loeffler Slant		Desoxycholate
Tetrathionate Br.		Blood + Menadione
Ethyl Violet Azide Broth		Lancefield Gr.
S.F. Medium		OTHER
Amm. Phos. Sl.		
Na Citrate Agar		

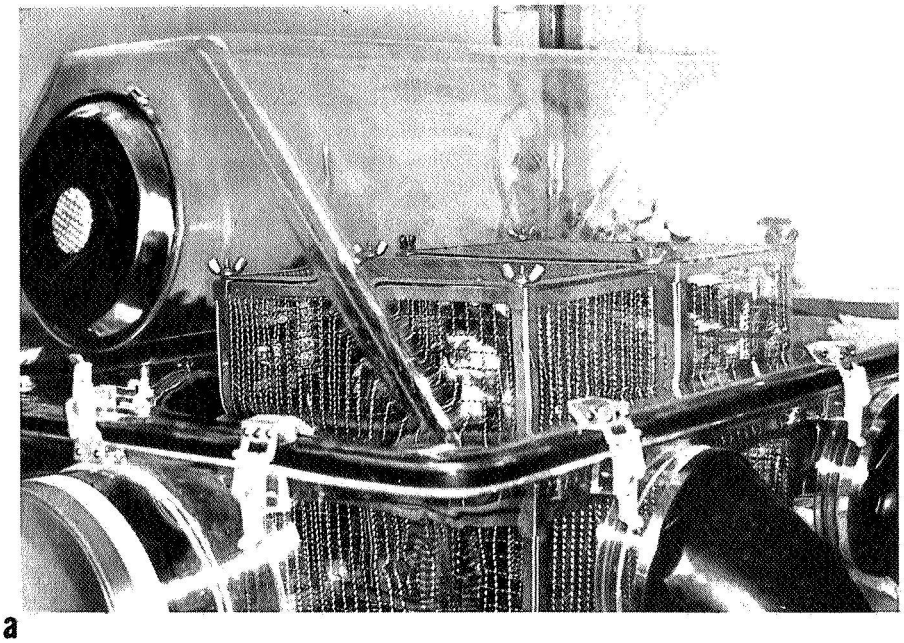
Figure 2

LEGEND FOR FIGURE 3

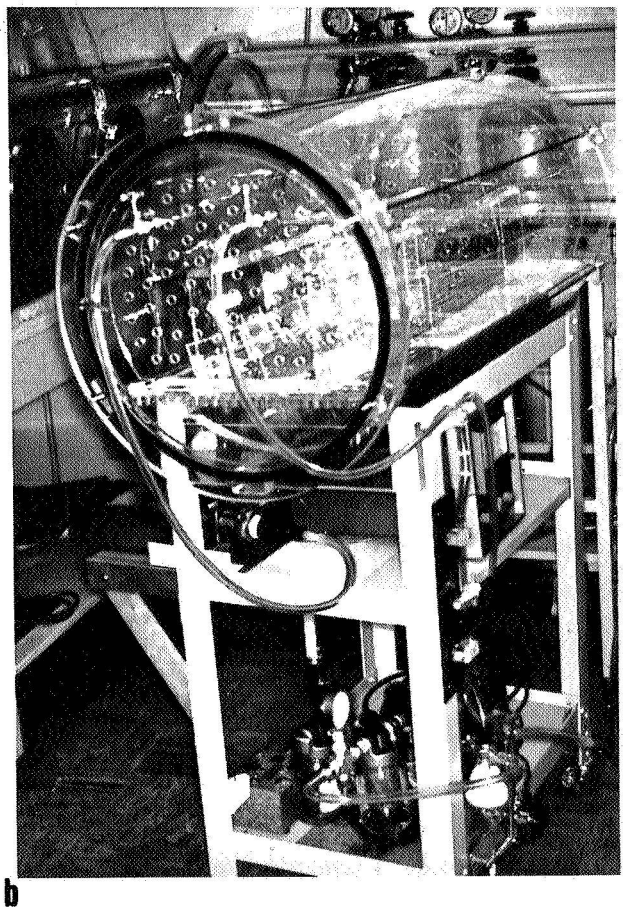
Germ-free plastic isolator and hypobaric pressure chamber.

a. Germ-free isolator containing two marmosets housed in separate cages. The isolator was used for prechamber and postchamber housing to study the animals at ambient conditions.

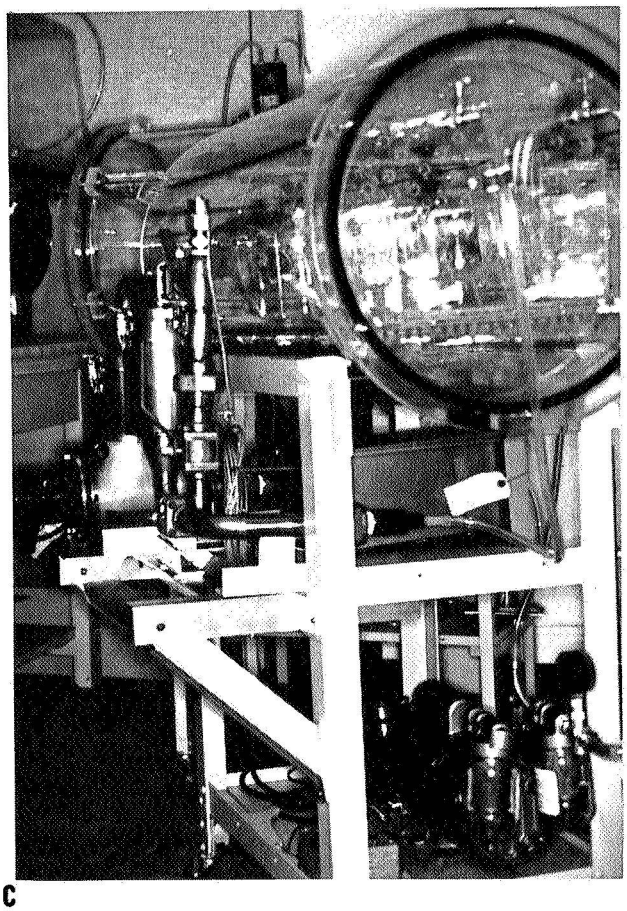
b & c. Opposing side views of the hypobaric pressure chamber, mobile stand, and regulatory devices.



a



b



c

Figure 3

LEGEND FOR FIGURE 4

Acrylic plastic cage racks for housing two marmosets in separate compartments in a hypobaric pressure chamber.

a. Removable partitioned rack with animal perches, vented end plates, center dividers, and attachable plastic watering troughs (WT), and vented food boxes (Fb).

b. Rack removed from chamber to show the floor of rack made from plastic strips to allow passage of animal wastes.

c & d. End and side view of the cage rack showing attachment of food and environmental control containers at the ends and center dividers with bolts and wing nuts. Two food boxes (Fb), one container of Baralyme (B), one container of a mixture of silica gel and Purafil (P), and two water troughs were placed in each compartment for one week housing.

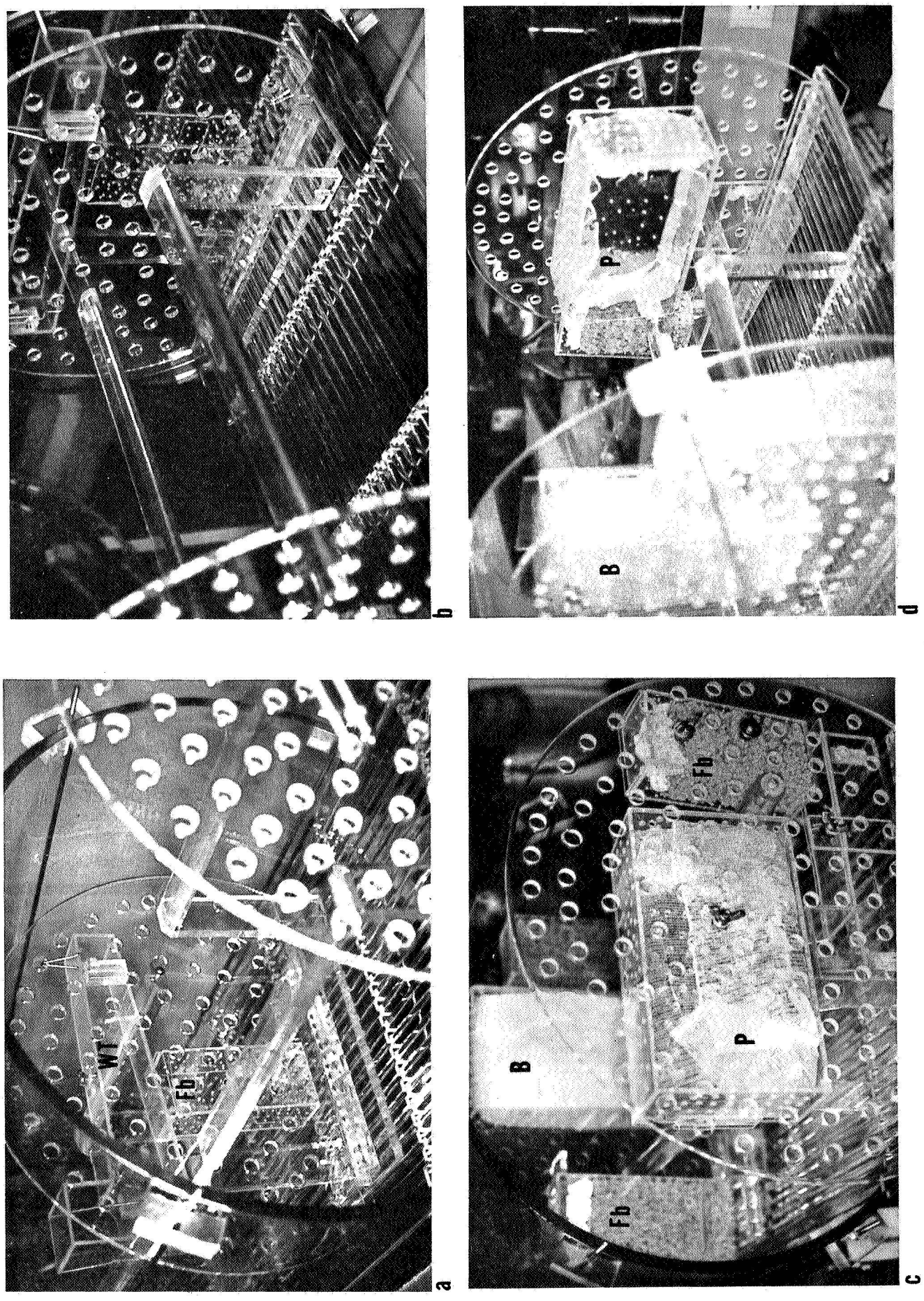


Figure 4

LEGEND FOR FIGURE 5

Units for recirculation of chamber atmosphere and oxygen monitoring.

a. Recirculation unit consisting of a magnetically driven squirrel cage blower (BL), canisters for chemical removal of CO_2 (-CO_2) and H_2O (-H_2O), partial by-pass or shut-off valves (V), and pilot strip (P).

b. Oxygen monitor (OM), oxygen gland assembly containing oxygen electrode (OG), pressure tubing for connecting recirculation unit to chamber (Tr), and to oxygen source (Ts), oxygen supply tank (O_2), and 70 per cent O_2/30 per cent N_2 supply tank (O_2N_2).

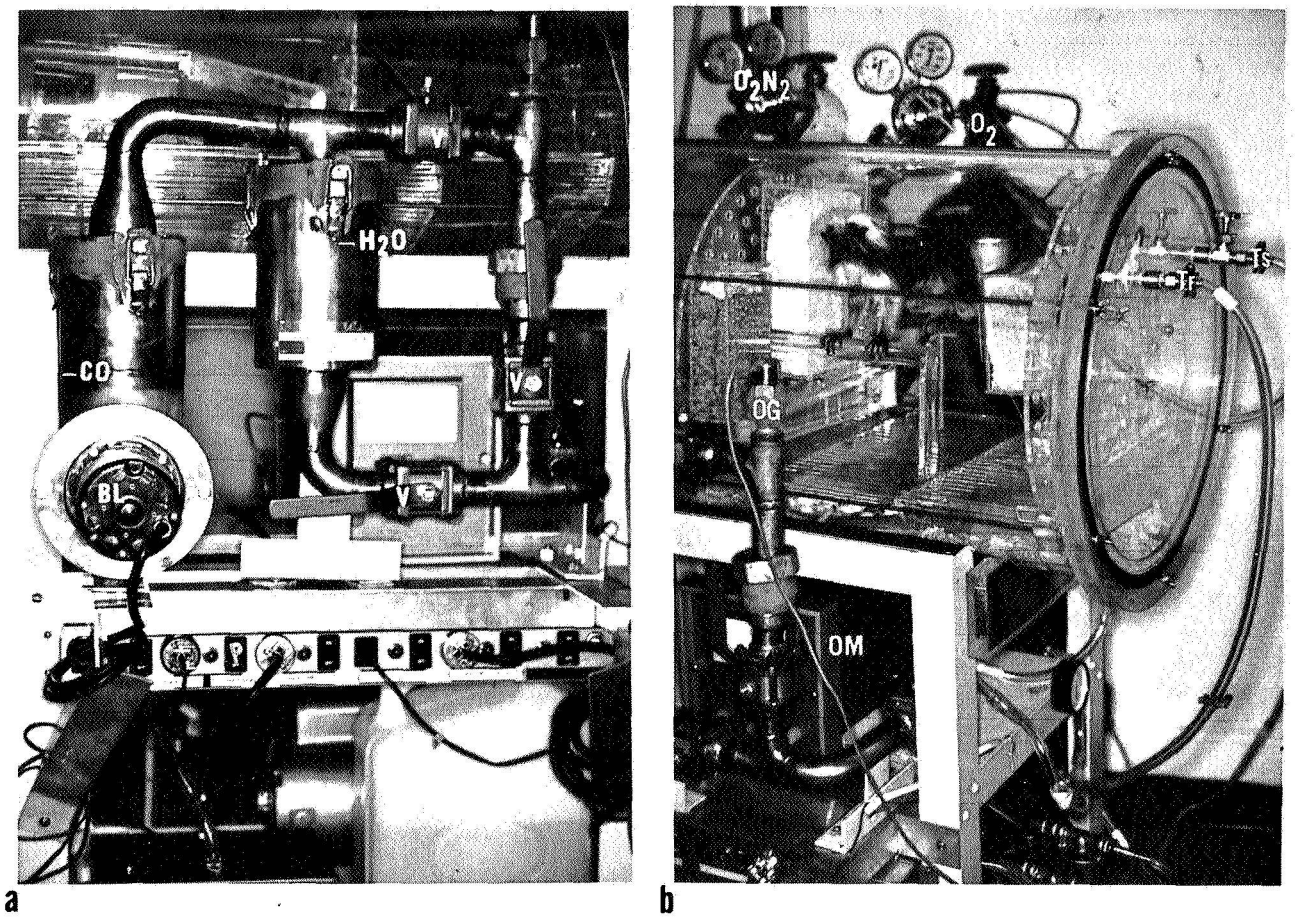


Figure 5

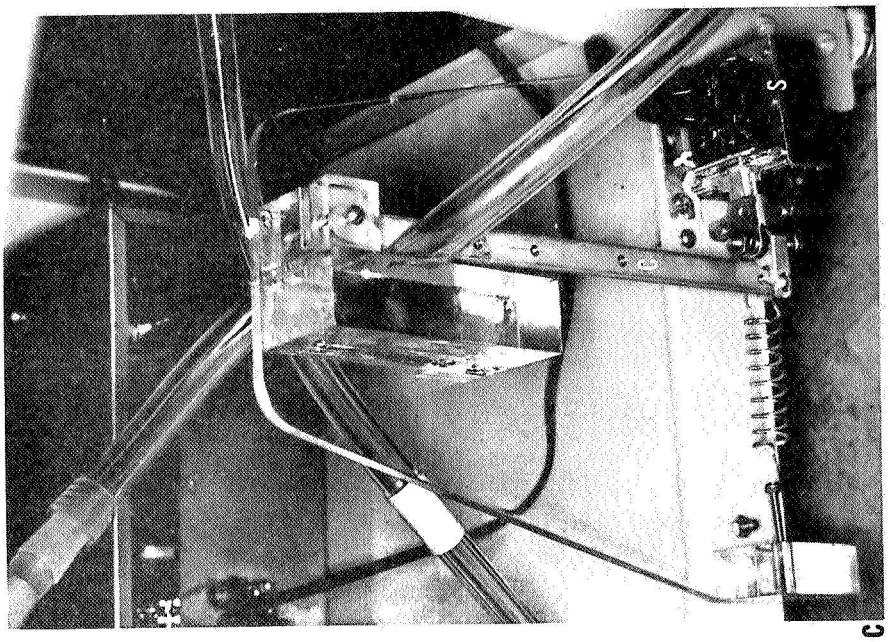
LEGEND FOR FIGURE 6

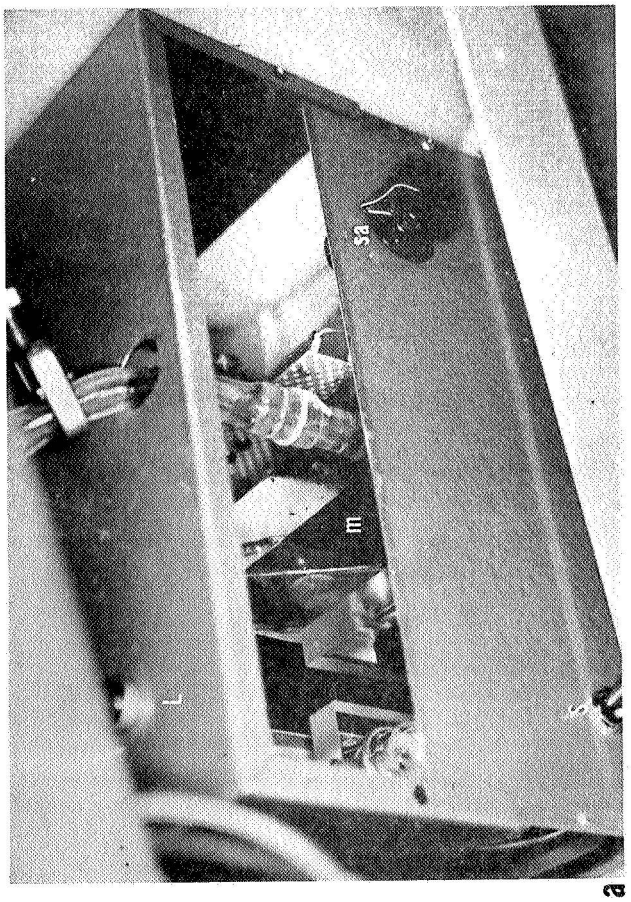
Components of pressure operated oxygen controller.

a. Complete housed unit with top partially opened to provide view of the control mechanism including on-off switch (S), indicator light (L), sensitivity adjustment (sa), "V"-shaped manometer in an acrylic plastic block (m) with one end connected to pressure tubing and clamp. The opposite ends of the manometer and tubing are attached to a glass Y located out of view above the unit. This allows pressure adjustments which do not affect the manometer when both connections are open. When one tube is clamped shut the manometer will reflect the pressure change.

b. Exposed view of control unit showing 10 V filament transformer (T), 6 to 8 volt auto light bulb (L), 10X microscope eyepiece (E), manometer (m), Raytheon photocell (P), amplifier (a), sensitivity control (sc), relay (R), and power supply (ps).

c. An oxygen valve consisting of a solenoid (S), tubing clamp (C), attached to and operating against a plastic block to open and close pressure tubing connecting oxygen supply tank to chamber. The valve is opened when a change in pressure moves the column of Hg to allow light passage and activation of the photocell. The impulse from the photocell is then amplified and relayed to the solenoid which releases the clamp.





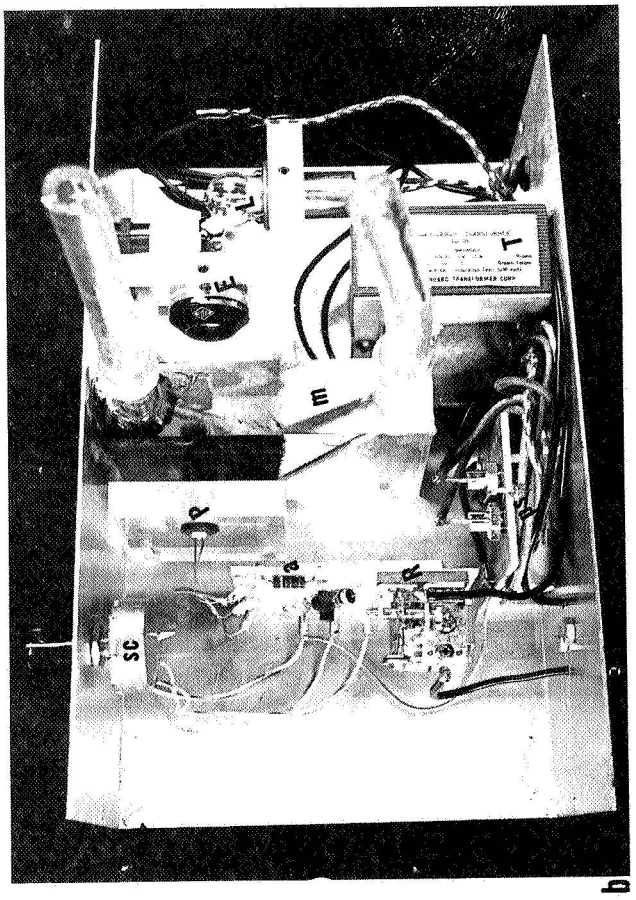


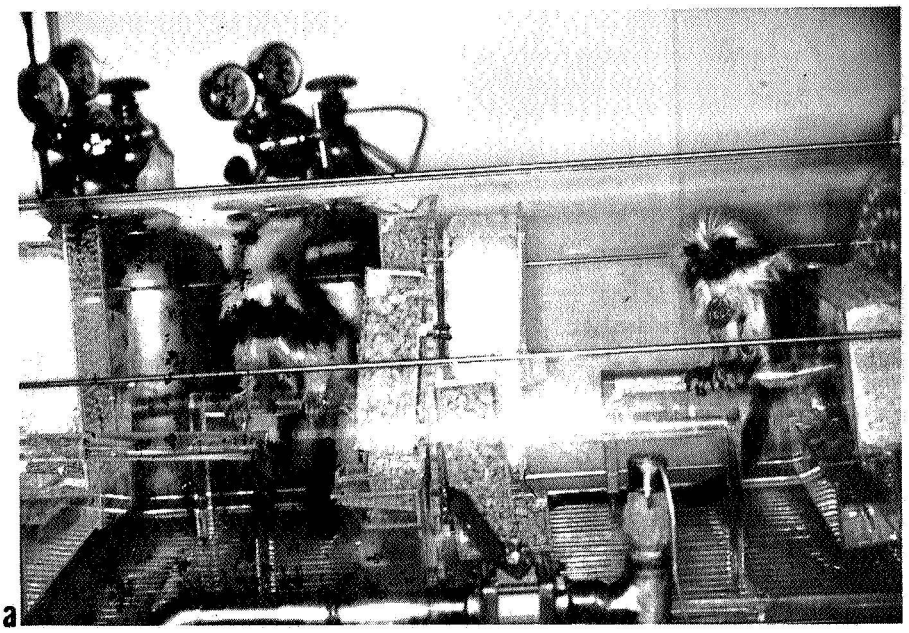
Figure 6

LEGEND FOR FIGURE 7

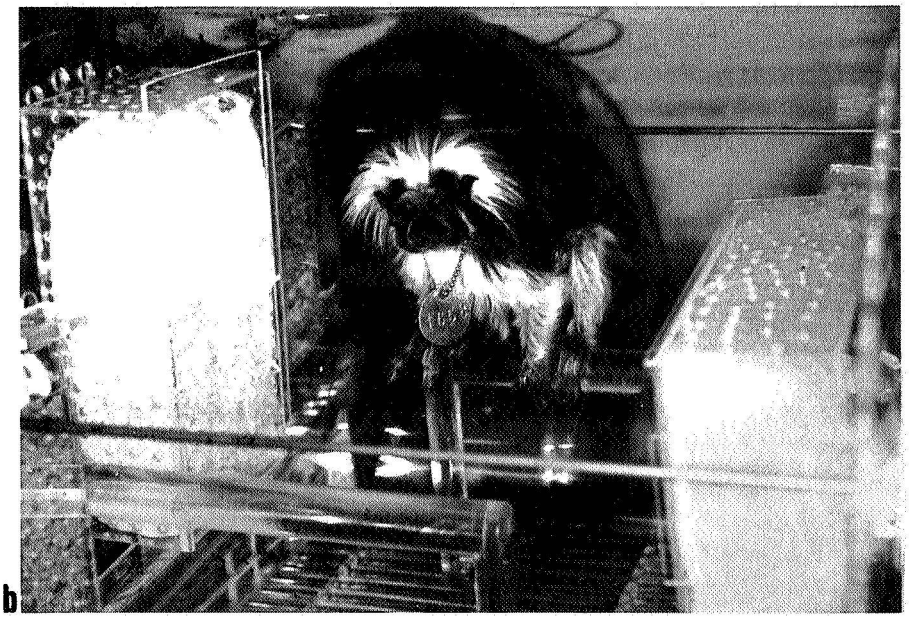
Marmosets housed in hypobaric pressure chamber in atmosphere of 70 per cent oxygen-30 per cent nitrogen at 5 PSI.

a. View of two marmosets housed in separate compartments within the chamber.

b. Enlargement of the above view showing the supplies needed to maintain one marmoset for seven days.



a



b

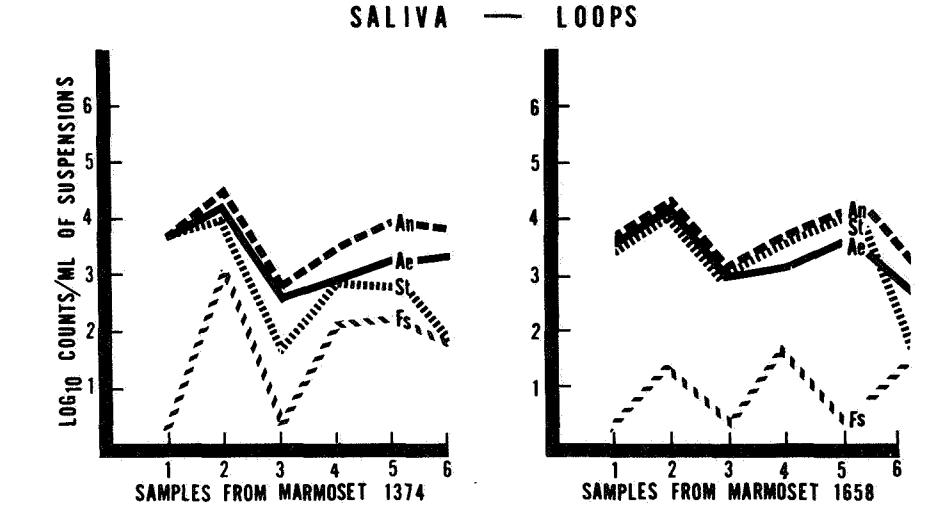
Figure 7

LEGEND FOR FIGURE 8

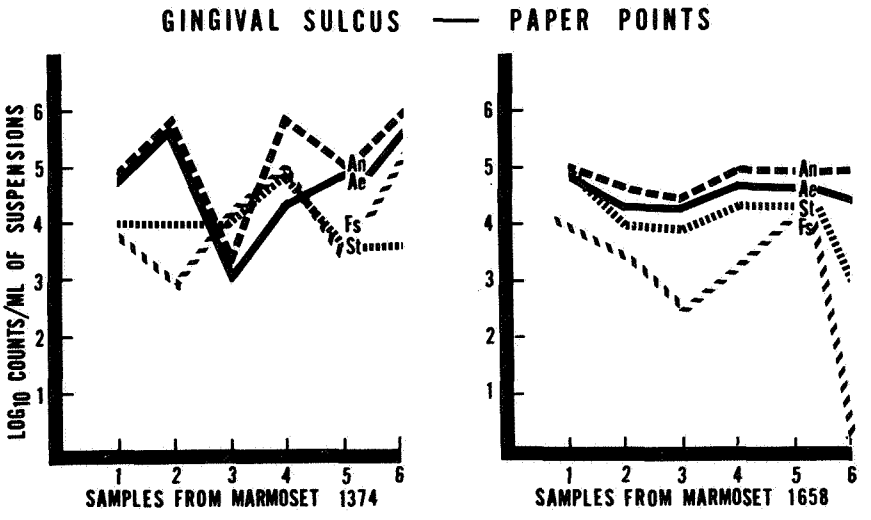
Comparison of counts ($\log_{10}$) of predominant cultivable microbes in oral specimens from marmosets.

a. Counts from saliva collected weekly by 2 mm wire loops.

b. Counts from the gingival sulcus collected by 3 mm paper points. Comparisons include total anaerobes (an), total aerobes (Ae), streptococci (St), and fusobacteria (Fs).


SALIVA — LOOPS
LOG10 COUNTS/ML OF SUSPENSIONS
An
Ae
St
Fs
SAMPLES FROM MARMOSET 1374
SAMPLES FROM MARMOSET 1658

a


GINGIVAL SULCUS — PAPER POINTS
LOG10 COUNTS/ML OF SUSPENSIONS
An
Ae
Fs
St
SAMPLES FROM MARMOSET 1374
SAMPLES FROM MARMOSET 1658

b

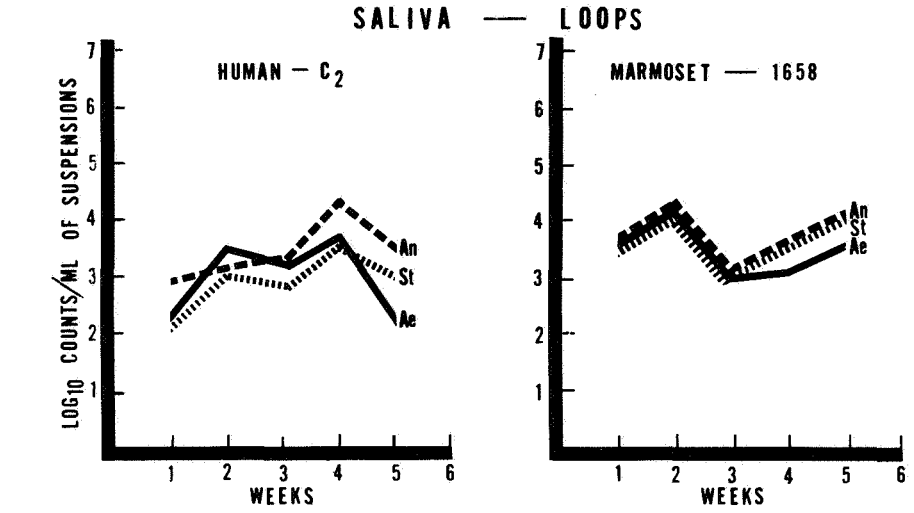
Figure 8

LEGEND FOR FIGURE 9

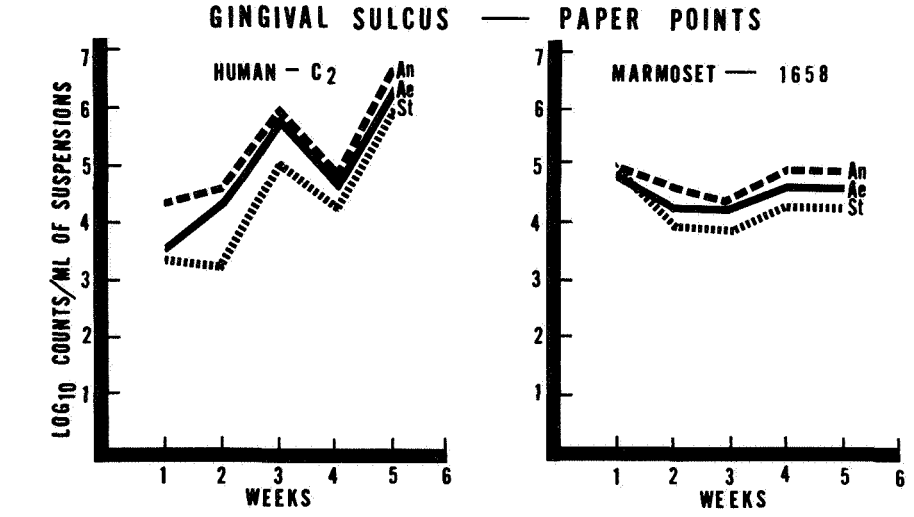
Comparison of counts ($\log_{10}$) of predominant cultivable microbes in oral specimens collected simultaneously from marmoset and human.

a. Counts from saliva collected by 2 mm wire loops.

b. Counts from the gingival sulcus collected by 3 mm paper points. Comparisons include total anaerobes (An), total aerobes (Ae), and streptococci (St).


SALIVA — LOOPS
HUMAN — C2
MARMOSET — 1658
LOG10 COUNTS/ML OF SUSPENSIONS
WEEKS
An
St
Ae

a


GINGIVAL SULCUS — PAPER POINTS
HUMAN — C2
MARMOSET — 1658
LOG10 COUNTS/ML OF SUSPENSIONS
WEEKS
An
Ae
St

b

Figure 9

LEGEND FOR FIGURE 10

Comparison of microbial counts from the saliva of marmosets prior to, during, and after isolation in a hypobaric pressure chamber.

The prechamber and postchamber values are medians of counts from four samples each. The one and two week chamber values are counts from one sample each. The microbial categories included are total anaerobes (An), total aerobes (Ae), and streptococci (St).

LEGEND FOR FIGURE 11

Comparison of microbial counts from the gingival sulcus of marmosets prior to, during, and after isolation in a hypobaric pressure chamber.

The prechamber and postchamber values are medians of counts from four samples each. The one and two week chamber values are counts from one sample each. The microbial categories included are total anaerobes (An), total aerobes (Ae), fusobacteria (F), streptococci (St), and bacteroides (B).

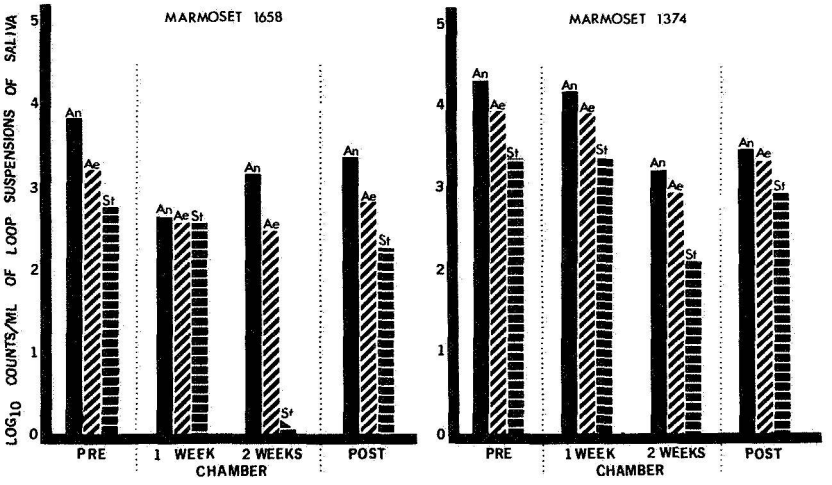


Figure 10

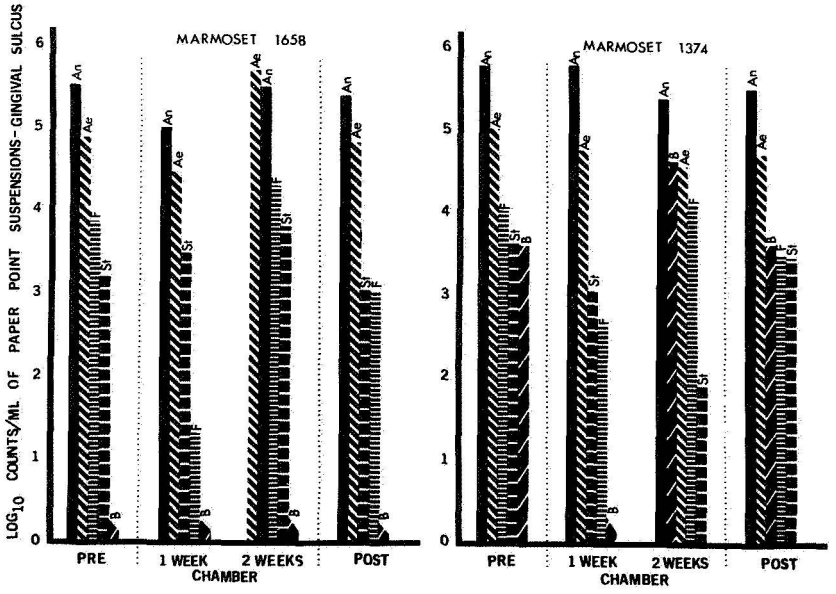


Figure 11

LEGEND FOR FIGURE 12

Comparison of microbial counts from oral swabs of marmosets prior to, during, and following isolation in a hypobaric pressure chamber.

The prechamber and postchamber values are medians of counts from four samples each. The one and two week chamber values are counts from one sample each. The microbial categories included are fusobacteria (F), staphylococci (S), bacteroides (B), and enterics (E).

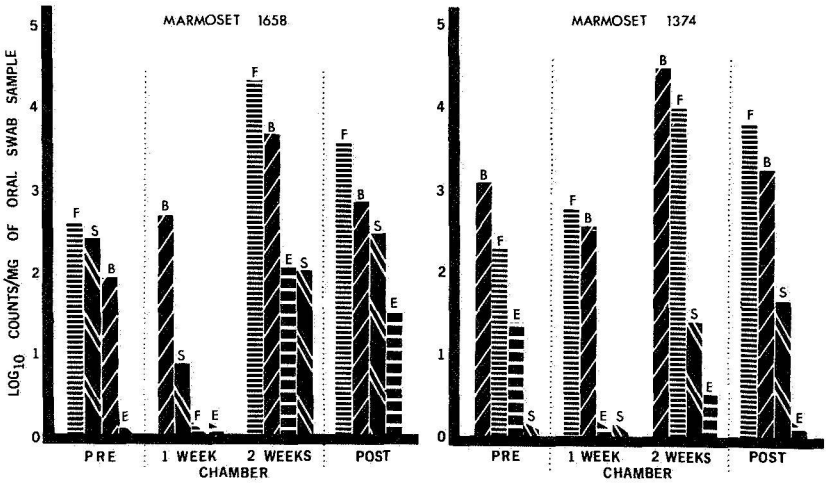

MARMOSET 1658
MARMOSET 1374
LOG10 COUNTS/MG OF ORAL SWAB SAMPLE
0
1
2
3
4
5
PRE
1 WEEK
2 WEEKS
POST
CHAMBER

Figure 12